

Mutagenic Evaluation of Compound FDA 71-36 (Calcium Propionate) 10/9/74

BB25

LBI PROJECT #02468

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MUTAGENIC EVALUATION OF
COMPOUND FDA 71-36
CALCIUM PROPIONATE

SUBMITTED TO
FOOD & DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
ROCKVILLE, MARYLAND

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EVALUATION SUMMARY

Compound FDA 71-36, calcium propionate, was not genetically active, either directly or in the presence of organ homogenates, in any of the in vitro assays employed in this evaluation.



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DATE: October 1, 1974

SPONSOR: Food and Drug Administration, Contract Number 223-74-2104

SUBJECT: Mutagenic Evaluation of Compound FDA 71-36

I. OBJECTIVE

The objective of this study was to assess the genetic activity of the test material in microbial assays with and without the addition of mammalian metabolic enzyme preparations.

II. MATERIALS

A. Test Material

Calcium propionate

Pfizer S-4181 #73211

B. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from liver, lung and testes of the following mammalian species: Mouse - ICR random bred adult males; rat - Sprague-Dawley adult males; and primate - Macaca mulatta adult males.

C. Indicator Organisms

The indicator organisms used for all tests are described below:

- Saccharomyces cerevisiae, strain D4: $\frac{\alpha}{a}$, $\frac{ade\ 2-2}{ade\ 2-1}$, $\frac{try\ 5-12}{try\ 5-27}$
- Salmonella typhimurium, strains:
 - TA-1535; hisG, uvrB, rfa (missense mutation)
 - TA-1537; hisC, uvrB, rfa (- frameshift mutation)
 - TA-1538; hisD, uvrB, rfa (+ frameshift mutation)

D. Reaction Mixture

The following reaction mixture was employed in the activation tests:



<u>Component</u>	<u>Final Concentration/ml</u>
1. TPN (sodium salt)	6 μ M
2. Isocitric acid	49 μ M
3. Tris buffer, pH 7.4	28 μ M
4. $MgCl_2$	1.7 μ M
5. Isocitric dehydrogenase	6.3 Units
6. Tissue homogenate or cell fraction	72 μ M

Components 1-4 were combined and frozen as a "core" reaction mixture to which the other components were added just prior to use.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1
POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

<u>ASSAY</u>	<u>CHEMICAL^a</u>	<u>SOLVENT</u>	<u>PROBABLE MUTAGENIC SPECIFICITY^b</u>
Non-activation	Ethylmethane sulfonate	Water or saline	BPS
	2-Nitrosofluorene	Dimethylsulfoxide ^c	FS
	Quinacrine or Quinacrine mustard	Water or saline	FS
Activation	Dimethylnitrosamine	Water or saline	BPS
	2-Acetylaminofluorene	Dimethylsulfoxide ^c	FS

^a Concentrations given in the Results Section.

^b BPS = base-pair substitution; FS = frameshift.

^c Previously shown to be non-mutagenic, see Appendix.

III. METHODS

A. Toxicity

The solubility, toxicity and doses for all chemicals were determined prior to screening.

Each chemical was tested for survival against strains TA-1537 and D4 over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival dose was determined from the survival curve and the 1/4 and 1/2 50% doses calculated.



If no toxicity was obtained for a chemical with a given strain, then a maximum dose of 5% (w/v) was used against the strain.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.

B. Plate Tests

Only three bacteria strains were tested in qualitative plate tests. In the non-activation procedure, approximately 10^9 cells of a log phase culture of the bacterial indicator strains were spread over the surface of a minimal plate, and a measured amount of the test chemical was placed in the center of the test plate. In activation tests, the test chemical was added to the cells, and an aliquot of the mixture was spread on the surface of the test plate. The reaction mixture (0.1 ml) plus tissue extract was then spotted on the surface of the plate. Positive and solvent controls were included. All plates were incubated at 37°C for four days and then scored. Each compound (Test, Positive Control and Solvent Control) was done in duplicate. The results were scored as + or -. Concentrations of the positive control compounds are listed in the Results Section.

C. Suspension Tests

1. Non-activation

Log-phase bacteria and stationary-phase yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1×10^9 cells/ml and 5×10^7 cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in 30 ml plastic tissue culture flasks. Cells plus appropriate volume(s) of the test chemical were added to the flasks to give a final volume of 2 ml. Solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the flasks were set in ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium. Samples from a 10^{-1} dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the non-activation tests except that the cell densities were increased approximately five-fold for working stock suspensions. Measured amounts of the test and



control chemicals plus 0.25 ml of the stock cell suspension were added to a 30 ml plastic tissue homogenate. All flasks (bacteria and yeast) were incubated at 37°C with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for non-activation tests.

D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

1. Mice

Male mice (sufficient to provide the necessary quantities of organs for testes, lung and liver homogenates) were killed by cranial blow, decapitated and bled. The three organs were immediately dissected from the animal using aseptic techniques and placed in ice-cold 0.25 M sucrose buffered with Tris at pH of 7.4. Upon collection of the desired quantity of organs, they were washed twice with fresh buffered sucrose and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies.

2. Rats

The same procedures as described for mice was used for this mammal.

3. Primates

The liver, lungs and testes were aseptically removed from freshly killed adult male rhesus (*M. mulatta*) monkeys. Each organ was cut into a number of pieces each sufficient for one week's studies. The tissues were labeled and frozen at -80°C until needed. Tissue homogenates and 9,000 x g supernatants were prepared as described for mice.

E. Data Recording and Reporting

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set was recorded, in ink, in bound data books. Information necessary for identification of the specific experiment as well as the presence of any contaminant micro-organisms was recorded with each set of plate counts. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated.

Frequencies were mechanically calculated and double checked. All data presented in the Results Section of this report consists of the actual sum of all raw data plate counts and only the frequencies are calculated figures.



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IV. SOLUBILITY PROPERTIES OF THE TEST COMPOUND

1. NAME OR DESCRIPTION OF TEST COMPOUND: FDA 71-36
CALCIUM PROPIONATE
2. TEST SOLVENT AND DESCRIPTION OF SOLUBILITY
OF THE TEST CHEMICAL UNDERTREATMENT
CONDITIONS: 0.067 M phosphate buffer, pH 7.4, was used as
the solvent for this compound, and the compound was soluble
under treatment conditions in all tests.
3. OTHER COMMENTS:

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V. TOXICITY AND DOSAGE DETERMINATIONS

COMPOUND FDA 71-36

	D4	TA-1537
	<u>Dose No.</u>	<u>% Concentration</u>
Range of concentrations of the test compound used to determine the 50% survival level	1	1
	2	2
	3	3
	4	4
	5	5
	<u>Dose No.</u>	<u>% Survival</u>
Survival Results	Control	100
Test Date: <u>7-8-74</u>	1	53
	2	22
	3	16
	4	6
	5	10
	<u>Dose</u>	<u>% Concentration</u>
Concentrations of the test chemical required for mutagenicity tests	Plate Test	0.5%
	1/4 50% Survival	0.25%
	1/2 50% Survival	0.5%
	Other 50%	-

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VI. NON-ACTIVATION PLATE TESTS

DATE: 8-30-74

Test	Compound	Concentration/plate	<u>TA-1535</u>		<u>TA-1537</u>		<u>TA-1538</u>	
			T-1	T-2	T-1	T-2	T-1	T-2
PC	EMS	0.05 ml undiluted chemical	+	+				
	QM	0.25 mg			+	+		
	NF	0.25 mg					+	+
SC	SALINE	-	-	-	-	-		
	DMSO	<10%					-	-

NOTE: PC = positive control
 SC = solvent control
 T-1 = trial 1
 T-2 = trial 2
 EMS = ethyl methanesulfonate
 QM = quinacrine mustard
 NF = nitrosofluorene
 DMSO = dimethyl sulfoxide
 (c) = contamination present

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NON-ACTIVATION PLATE TESTS

DATE: 8-30-74

Test	Compound	Concentration	<u>TA-1535</u>		<u>TA-1537</u>		<u>TA-1538</u>	
			T-1	T-2	T-1	T-2	T-1	T-2
TC	FDA 71-36	0.50%	-	-	-	-	-	-

NOTE: TC = test compound
T-1 = trial 1
T-2 = trial 2

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VII. ACTIVATION PLATE TESTS

SPECIES: MOUSE

DATE: 8-30-74

Test	Organ	Compound	Concentration/plate	TA-1535		TA-1537		TA-1538	
				T-1	T-2	T-1	T-2	T-1	T-2
PC	Li	DMNA	25 μ moles	+	+				
		AAF	1.25 mg			+	+	+	+
	Lu	DMNA	25 μ moles	+/-	+/-				
		AAF	1.25 mg			-	-	-	-
	T	DMNA	25 μ moles	-	-				
		AAF	1.25 mg			-	-	-	-
SC	-	DMNA	25 μ moles	-	-				
	-	AAF	1.25 mg			-	-	-	-
	-	Saline	-	-	-				
	-	DMSO	<10%			-	-	-	-

NOTE: PC = positive control
 SC = solvent and chemical controls
 AAF = 2-acetylaminofluorene
 DMNA = dimethylnitrosamine
 Li = liver
 Lu = lung

T = testes
 T-1 = trial 1
 T-2 = trial 2
 DMSO = dimethyl sulfoxide
 (c) = contamination present

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ACTIVATION PLATE TESTS

SPECIES: MOUSE

DATE: 8-30-74

Test	Organ	Compound	Concentration	<u>TA-1535</u>		<u>TA-1537</u>		<u>TA-1538</u>	
				T-1	T-2	T-1	T-2	T-1	T-2
TC	Li	FDA 71-36	0.50%	-	-	-	-	-	-
	Lu	FDA 71-36	0.50%	-	-	-	-	-	-
	T	FDA 71-36	0.50%	-	-	-	-	-	-

NOTE: TC = test compound
 Li = liver
 Lu = lung
 T = testes
 T-1 = trial 1
 T-2 = trial 2

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ACTIVATION PLATE TESTS

SPECIES: RAT

DATE: 8-30-74

Test	Organ	Compound	Concentration/plate	TA-1535		TA-1537		TA-1538	
				T-1	T-2	T-1	T-2	T-1	T-2
PC	Li	DMNA	25 μ moles	+	+				
		AAF	1.25 mg			+	+	+	+
	Lu	DMNA	25 μ moles	-	-				
		AAF	1.25 mg			-	-	-	-
	T	DMNA	25 μ moles	-	-				
		AAF	1.25 mg			-	-	-	-
SC	-	DMNA	25 μ moles	-	-				
	-	AAF	1.25 mg			-	-	-	-
	-	Saline	-	-	-				
	-	DMSO	<10%			-	-	-	-

NOTE: PC = positive control
 SC = solvent and chemical controls
 AAF = 2-acetylaminofluorene
 DMNA = dimethylnitrosamine
 Li = liver
 Lu = lung

T = testes
 T-1 = trial 1
 T-2 = trial 2
 DMSO = dimethyl sulfoxide
 (c) = contamination present

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ACTIVATION PLATE TESTS

SPECIES: RAT

DATE: 8-30-74

Test	Organ	Compound	Concentration	<u>TA-1535</u>		<u>TA-1537</u>		<u>TA-1538</u>	
				T-1	T-2	T-1	T-2	•T-1	T-2
TC	Li	FDA 71-36	0.50%	-	-	-	-	-	-
	Lu	FDA 71-36	0.50%	-	-	-	-	-	-
	T	FDA 71-36	0.50%	-	-	-	-	-	-

NOTE: TC = test compound
 Li = liver
 Lu = lung
 T = testes
 T-1 = trial 1
 T-2 = trial 2

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ACTIVATION PLATE TESTS

SPECIES: MONKEY

DATE: 8-30-74

Test	Organ	Compound	Concentration/plate	TA-1535		TA-1537		TA-1538	
				T-1	T-2	T-1	T-2	T-1	T-2
PC	Li	DMNA	25 μ moles	+	+				
		AAF	1.25 mg			+	+	+	+
	Lu	DMNA	25 μ moles	+/-	+/-				
		AAF	1.25 mg			-	-	-	-
	T	DMNA	25 μ moles	-	-				
		AAF	1.25 mg			-	-	-	-
SC	-	DMNA	25 μ moles	-	-				
	-	AAF	1.25 mg			-	-	-	-
	-	Saline	-	-	-				
	-	DMSO	<10%			-	-	-	-

NOTE: PC = positive control
SC = solvent and chemical controls
AAF = 2-acetylaminofluorene
DMNA = dimethylnitrosamine
Li = liver
Lu = lung

T = testes
T-1 = trial 1
T-2 = trial 2
DMSO = dimethyl sulfoxide
(c) = contamination present

Project No. 02468

ACTIVATION PLATE TESTS

SPECIES: MONKEY

DATE: 8-30-74

Test	Organ	Compound	Concentration	<u>TA-1535</u>		<u>TA-1537</u>		<u>TA-1538</u>	
				T-1	T-2	T-1	T-2	T-1	T-2
TC	Li	FDA 71-36	0.50%	-	-	-	-	-	-
	Lu	FDA 71-36	0.50%	-	-	-	-	-	-
	T	FDA 71-36	0.50%	-	-	-	-	-	-

NOTE: TC = test compound
 Li = liver
 Lu = lung
 T = testes
 T-1 = trial 1
 T-2 = trial 2

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VIII. NON-ACTIVATION SUSPENSION TESTS
WITH SALMONELLA INDICATOR STRAINS:
POSITIVE AND SOLVENT CONTROL RESULTS

DATE: 8-16-74

Test	Indicator Strain	Compound	Concentration	Total Cells/ ml x 10 ⁸	his+ Revertants/ ml	his+ Revertants/10 ⁸ Survivors
PC	TA-1535	EMS	0.05 %	10.31	2,628	254.90
	TA-1537	QM	0.01 mg/ml	2.79	59	21.15
	TA-1538	NF	1.25 mg/ml	2.66	43	16.17
SC	TA-1535	SALINE	-	8.75	9	1.03
	TA-1537	SALINE	-	3.70	12	3.24
	TA-1538	DMSO	-	5.17	9	1.74

NOTE: PC = positive control
 SC = solvent control
 EMS = ethyl methanesulfonate
 QM = quinacrine mustard
 NF = nitrofluorene
 DMSO = dimethyl sulfoxide

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BIONETICS

NON-ACTIVATION SUSPENSION TESTS
WITH SALMONELLA INDICATOR STRAINS

DATE: 8-16-74

Test	Indicator Strain	Compound	Concentration	Total Cells/ ml x 10 ⁸	his+ Revertants/ ml	his+ Revertants/10 ⁸ Survivors
TC-H	TA-1535	FDA 71-36	0.50%	2.13 (24)	1	0.47
TC-L	TA-1535	FDA 71-36	0.25%	2.78 (32)	2	0.72
TC-H	TA-1537	FDA 71-36	0.50%	2.72 (74)	9	3.31
TC-L	TA-1537	FDA 71-36	0.25%	3.38 (91)	8	2.37
TC-H	TA-1538	FDA 71-36	0.50%	No data recorded		
TC-L	TA-1538	FDA 71-36	0.25%	7.07 (137)	14	1.98

NOTE: TC-H = test compound high dose
TC-L = test compound low dose
() = percent survival

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NON-ACTIVATION SUSPENSION TESTS
WITH SALMONELLA INDICATOR STRAINS:
POSITIVE AND SOLVENT CONTROL RESULTS

(Repeated Dose) •

DATE: 9-27-74

Test	Indicator Strain	Compound	Concentration	Total Cells/ ml x 10 ⁸	his+ Revertants/ ml	his+ Revertants/10 ⁸ Survivors
PC	TA-1535	EMS	0.05 %			
	TA-1537	QM	0.01 mg/ml			
	TA-1538	NF	1.25 mg/ml	4.86	216	44.44
SC	TA-1535	SALINE	-			
	TA-1537	SALINE	-			
	TA-1538	DMSO	-	3.53	25	7.08

NOTE: PC = positive control
 SC = solvent control
 EMS = ethyl methanesulfonate
 QM = quinacrine mustard
 NF = nitrosofluorene
 DMSO = dimethyl sulfoxide
 (c) = contamination present

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NON-ACTIVATION SUSPENSION TESTS
WITH SALMONELLA INDICATOR STRAINS

(Repeated Dose)

DATE: 9-27-74

Test	Indicator Strain	Compound	Concentration	Total Cells/ mlx10 ⁸	his+ Revertants/ ml	his+ Revertants/10 ⁸ Survivors
TC	TA-1535		H			
TC	TA-1535		L			
TC	TA-1537		H			
TC	TA-1537		L			
TC	TA-1538	FDA 71-36	H 0.50%	3.08 (87)	19	6.17
TC	TA-1538		L			

NOTE: TC = test compound
H = high dose
L = low dose
(c) = contamination present
() = percent survival

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IX. ACTIVATION SUSPENSION TESTS
WITH SALMONELLA INDICATOR STRAINS:
POSITIVE AND SOLVENT CONTROL RESULTS

SPECIES: MOUSE

DATE: 7-24-74

Strain TA-1535

Test	Organ	Compound	Concentration	Total Cells/ mlx10 ⁸	his+ Revertants/ ml	his+ Revertants/10 ⁸ Survivors
PC	Li	DMNA	100 μ moles/ml	5.71	2,233	391.07
	Lu	DMNA	100 μ moles/ml	4.99	49	9.82
	T	DMNA	100 μ moles/ml	7.25	5	0.69
SC	-	DMNA	100 μ moles/ml	10.55	11	1.04
	-	SALINE	-	5.80	11	1.90

DATE: 7-25-74

Strain TA-1537

Test	Organ	Compound	Concentration	Total Cells/ mlx10 ⁸	his+ Revertants/ ml	his+ Revertants/10 ⁸ Survivors
PC	Li	AAF	1.25 mg/ml	2.67	45	16.85
	Lu	AAF	1.25 mg/ml	1.85	11	5.95
	T	AAF	1.25 mg/ml	2.90	16	5.52
SC	-	AAF	1.25 mg/ml	2.11	6	2.84
	-	DMSO	-	1.83	11	6.01

DATE: 7-26-74

Strain TA-1538

Test	Organ	Compound	Concentration	Total Cells/ mlx10 ⁸	his+ Revertants/ ml	his+ Revertants/10 ⁸ Survivors
PC	Li	AAF	1.25 mg/ml	2.36	53	22.46
	Lu	AAF	1.25 mg/ml	2.40	16	6.67
	T	AAF	1.25 mg/ml	2.35	10	4.26
SC	-	AAF	1.25 mg/ml	2.75	13	4.73
	-	DMSO	-	2.26 (c)	6	2.65

NOTE: PC = positive control
 SC = solvent and chemical controls
 AAF = 2-acetylaminofluorene
 DMNA = dimethylnitrosamine
 Li = liver
 Lu = lung
 T = testes
 DMSO = dimethyl sulfoxide

(c) = contamination present

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ACTIVATION SUSPENSION TESTS
WITH SALMONELLA INDICATOR STRAINS

SPECIES: MOUSE

DATE: 7-24-74

Strain TA-1535

Test	Organ	Compound	Concentration	Total Cells/ mlx10 ⁸	his+ Revertants/ ml	his+ Revertants/10 ⁸ Survivors
TC	Li	FDA 71-36	H	7.30(126)	5	0.68
		FDA 71-36	L	14.30(247)	7	0.49
	Lu	FDA 71-36	H	8.61(148)	3	0.35
		FDA 71-36	L	6.58(113)	2	0.30
	T	FDA 71-36	H	6.97(120)	3	0.43
		FDA 71-36	L	7.24(125)	10	1.38

DATE: 7-25-74

Strain TA-1537

TC	Li	FDA 71-36	H	2.81(154)	11	3.91
		FDA 71-36	L	3.07(c)(168)	13	4.23
	Lu	FDA 71-36	H	1.92(105)	11	5.73
		FDA 71-36	L	4.13(226)	11	2.66
	T	FDA 71-36	H	2.94(161)	8	2.72
		FDA 71-36	L	2.68(146)	8	2.99

DATE: 7-26-74

Strain TA-1538

TC	Li	FDA 71-36	H	3.43(152)	6	1.75
		FDA 71-36	L	3.76(166)	12	3.19
	Lu	FDA 71-36	H	3.85(170)	12	3.12
		FDA 71-36	L	3.42(151)	5	1.46
	T	FDA 71-36	H	4.61(204)	6	1.30
		FDA 71-36	L	4.63(205)	8	1.73

NOTES: H = high dose
L = low dose
TC = test compound
Li = liver
Lu = lung
T = testes
(c) = contamination present
() = percent survival

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ACTIVATION SUSPENSION TESTS
WITH SALMONELLA INDICATOR STRAINS:
POSITIVE AND SOLVENT CONTROL RESULTS

SPECIES: RAT

DATE: 8-7-74

Strain TA-1535

Test	Organ	Compound	Concentration	Total Cells/ mlx10 ⁸	his+ Revertants/ ml	his+ Revertants/10 ⁸ Survivors
PC	Li	DMNA	100 μ moles/ml	6.54	4,269	652.75
	Lu	DMNA	100 μ moles/ml	6.46	12	18.46
	T	DMNA	100 μ moles/ml	9.37	3	0.32
SC	-	DMNA	100 μ moles/ml	5.74	11	1.92
	-	SALINE	-	4.17	8	1.92

DATE: 8-8-74

Strain TA-1537

Test	Organ	Compound	Concentration	Total Cells/ mlx10 ⁸	his+ Revertants/ ml	his+ Revertants/10 ⁸ Survivors
PC	Li	AAF	1.25 mg/ml	1.25	38	30.40
	Lu	AAF	1.25 mg/ml	6.50	33	5.08
	T	AAF	1.25 mg/ml	4.53	18	3.97
SC	-	AAF	1.25 mg/ml	5.62	6	1.07
	-	DMSO	-	5.88	9	1.53

DATE: 8-9-74

Strain TA-1538

Test	Organ	Compound	Concentration	Total Cells/ mlx10 ⁸	his+ Revertants/ ml	his+ Revertants/10 ⁸ Survivors
PC	Li	AAF	1.25 mg/ml	2.84	165	58.01
	Lu	AAF	1.25 mg/ml	3.95	20	5.06
	T	AAF	1.25 mg/ml	5.09	26	5.11
SC	-	AAF	1.25 mg/ml	3.88	10	2.58
	-	DMSO	-	4.21	8	1.90

NOTE: PC = positive control
 SC = solvent and chemical controls
 AAF = 2-acetylaminofluorene
 DMNA = dimethylnitrosamine
 Li = liver
 Lu = lung
 T = testes
 DMSO = dimethyl sulfoxide

(c) = contamination present

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ACTIVATION SUSPENSION TESTS
WITH SALMONELLA INDICATOR STRAINS

SPECIES: RAT

DATE: 8-7-74

Strain TA-1535

Test	Organ	Compound	Concentration	Total Cells/ mlx10 ⁸	his+ Revertants/ ml	his+ Revertants/10 ⁸ Survivors
TC	Li	FDA 71-36	H	4.84(116)	8	1.65
		FDA 71-36	L	4.85(116)	27	5.57
	Lu	FDA 71-36	H	3.51(84)	9	2.56
		FDA 71-36	L	7.61(182)	18	2.37
	T	FDA 71-36	H	5.87(141)	13	2.21
		FDA 71-36	L	5.94(142)	9	1.52

DATE: 8-8-74

Strain TA-1537

TC	Li	FDA 71-36	H	6.71(114)	6	0.89
		FDA 71-36	L	5.23(89)	28	5.35
	Lu	FDA 71-36	H	4.18(71)	6	1.44
		FDA 71-36	L	3.59(61)	17	4.74
	T	FDA 71-36	H	4.64(79)	15	3.23
		FDA 71-36	L	5.89(100)	16	2.72

DATE: 8-9-74

Strain TA-1538

TC	Li	FDA 71-36	H	5.26(125)	9	1.71
		FDA 71-36	L	2.21(50)	8	3.62
	Lu	FDA 71-36	H	4.88(116)	5	1.02
		FDA 71-36	L	3.72(88)	2	0.54
	T	FDA 71-36	H	3.26(77)	18	5.52
		FDA 71-36	L	3.97(94)	18	4.53

NOTES: H = high dose () = percent survival
 L = low dose
 TC = test compound
 Li = liver
 Lu = lung
 T = testes
 (c) = contamination present

Project No. 02468



BIONETICS

ACTIVATION SUSPENSION TESTS
WITH SALMONELLA INDICATOR STRAINS:
POSITIVE AND SOLVENT CONTROL RESULTS

SPECIES: MONKEY

DATE: 9-5-74

Strain TA-1535

Test	Organ	Compound	Concentration	Total Cells/ mlx10 ⁸	his+ Revertants/ ml	his+ Revertants/10 ⁸ Survivors
PC	Li	DMNA	100 μ moles/ml	6.49	3,117	480.28
	Lu	DMNA	100 μ moles/ml	6.03	19	3.15
	T	DMNA	100 μ moles/ml	5.63	14	2.49
SC	-	DMNA	100 μ moles/ml	6.40	23	3.59
	-	SALINE	-	5.11	10	1.96

DATE: 9-17-74

Strain TA-1537

Test	Organ	Compound	Concentration	Total Cells/ mlx10 ⁸	his+ Revertants/ ml	his+ Revertants/10 ⁸ Survivors
PC	Li	AAF	1.25 mg/ml	5.47	84	15.36
	Lu	AAF	1.25 mg/ml	5.64	17	3.01
	T	AAF	1.25 mg/ml	4.57	19	4.16
SC	-	AAF	1.25 mg/ml	5.67	40	7.05
	-	DMSO	-	6.20	44	7.10

DATE: 9-27-74

Strain TA-1538

Test	Organ	Compound	Concentration	Total Cells/ mlx10 ⁸	his+ Revertants/ ml	his+ Revertants/10 ⁸ Survivors
PC	Li	AAF	1.25 mg/ml	6.68	305	45.66
	Lu	AAF	1.25 mg/ml	5.09	52	10.22
	T	AAF	1.25 mg/ml	5.15	33	6.41
SC	-	AAF	1.25 mg/ml	5.07	40	7.89
	-	DMSO	-	4.72	40	8.47

NOTE: PC = positive control
 SC = solvent and chemical controls (c) = contamination present
 AAF = 2-acetylaminofluorene
 DMNA = dimethylnitrosamine
 Li = liver
 Lu = lung
 T = testes
 DMSO = dimethyl sulfoxide

Project No. 02468



BIONETICS

ACTIVATION SUSPENSION TESTS
WITH SALMONELLA INDICATOR STRAINS

SPECIES: MONKEY

DATE: 9-5-74

Strain TA-1535

Test	Organ	Compound	Concentration	Total Cells/ mlx10 ⁸	hist+ Revertants/ ml	hist+ Revertants/10 ⁸ Survivors
TC	Li	FDA 71-36	H	6.28(123)	16	2.55
		FDA 71-36	L	5.36(105)	18	3.36
	Lu	FDA 71-36	H	4.81(94)	18	3.74
		FDA 71-36	L	4.61(90)	14	3.04
	T	FDA 71-36	H	5.37(105)	38	7.10
		FDA 71-36	L	5.80(114)	14	2.41

DATE: 9-17-74

Strain TA-1537

TC	Li	FDA 71-36	H	8.55(138)	42	4.91
		FDA 71-36	L	6.54(105)	55	8.41
	Lu	FDA 71-36	H	7.15(115)	32	4.48
		FDA 71-36	L	7.56(122)	29	3.84
	T	FDA 71-36	H	6.30(102)	89	14.13
		FDA 71-36	L	7.05(114)	45	6.38

DATE: 9-27-74

Strain TA-1538

TC	Li	FDA 71-36	H	1.51(32)	10	6.62
		FDA 71-36	L	2.16(46)	15	6.94
	Lu	FDA 71-36	H	1.20(25)	12	10.00
		FDA 71-36	L	2.70(57)	19	7.04
	T	FDA 71-36	H	2.02(43)	14	6.93
		FDA 71-36	L	3.86(82)	19	4.92

NOTES: H = high dose
L = low dose
TC = test compound
Li = liver
Lu = lung
T = testes
(c) = contamination present
() = percent survival

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BIONETICS

Litton

X. NON-ACTIVATION SUSPENSION TESTS
WITH SACCHAROMYCES INDICATOR STRAIN D4

DATE: 7-29-74

Strain D4

Test	Compound	Concentration	Total Population Screened ^a	Number of Convertants ^b		Convertants Per 10 ⁵ Survivors	
				Ade ⁺	Try ⁺	Ade ⁺	Try ⁺
PC	EMS	1.0 %	4.71	479	315	101.70	66.88
SC	Saline	-	4.39	117	14	26.65	3.19

JTE: PC = positive control
 SC = solvent control
 EMS = ethyl methanesulfonate
 a = number x 10⁵
 b = number at 10⁻¹ dilution

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BIONETICS

NON-ACTIVATION SUSPENSION TESTS
WITH SACCHAROMYCES INDICATOR STRAIN D4

DATE: 7-29-74

Strain D4							
Test	Compound	Concentration	Total Population Screened ^a	Number Convertants ^b		Convertants Per 10 ⁵ Survivors	
				Ade ⁺	Try ⁺	Ade ⁺	Try ⁺
TC	FDA 71-36	H	5.17 (130)	104	12	20.12	2.32
	FDA 71-36	L	4.08 (93)	69	12	16.91	2.94

NOTE: TC = test compound
H = high dose
L = low dose
a = number x 10⁵
b = number at 10⁻¹ dilution
() = percent survival

Project No. 02468



BIONETICS

XI. ACTIVATION SUSPENSION TESTS
WITH SACCHAROMYCES INDICATOR STRAIN D4:
POSITIVE AND SOLVENT CONTROL RESULTS

SPECIES: MOUSE

DATE: 7-29-74

Strain D4

Test	Organ	Compound	Concentration	Total Population Screened ^a	Number of Convertants ^b		Convertants Per 10 ⁵ Survivors	
					Ade ⁺	Try ⁺	Ade ⁺	Try ⁺
PC	Li	DMNA	150 μ moles/ml	2.90	237	67	81.72	23.10
	Lu	DMNA	150 μ moles/ml	3.23	68	27	21.05	8.36
	T	DMNA	150 μ moles/ml	3.38	61	11	18.05	3.25
SC	-	DMNA	150 μ moles/ml	2.89	42	13	14.53	4.50
	-	SALINE	-	2.75	89	6	32.36	2.18

NOTE: PC = positive control
 SC = solvent and chemical controls
 DMNA = dimethylnitrosamine
 Li = liver
 Lu = lung
 T = testes

a = number x 10⁵
 b = number at 10⁻¹ dilution
 (c) = contamination present

Project No. 02468



BIONETICS

Litton

ACTIVATION SUSPENSION TESTS
WITH SACCHAROMYCES INDICATOR STRAIN D4

SPECIES: MOUSE

DATE: 7-29-74

Strain D4

Test	Organ	Compound	Concentration	Total Population Screened ^a	Number of Conyertants ^b		Conyertants Per 10 ⁵ Survivors	
					Ade ⁺	Try ⁺	Ade ⁺	Try ⁺
TC	Li	FDA 71-36	H	3.54 (129)	59	11	16.67	3.11
		FDA 71-36	L	4.58 (167)	79	10	17.25	2.18
	Lu	FDA 71-36	H	3.75 (136)	113	13	30.13	3.47
		FDA-71-36	L	4.45 (162)	87	13	19.55	2.92
	T	FDA 70-36	H	3.66 (133)	103	9	28.14	2.46
		FDA 71-36	L	3.39 (123)	79	6	23.30	1.77

NOTE:

TC = test compound

H = high dose

L = low dose

Li = liver

Lu = lung

T = testes

a = number x 10⁵

b = number at 10⁻¹ dilution

(c) = contamination present

{ } = percent survival

Project No. 02468



BIONETICS

ACTIVATION SUSPENSION TESTS
WITH SACCHAROMYCES INDICATOR STRAIN D4

SPECIES: RAT

DATE: 8-5-74

Strain D4

Test	Organ	Compound	Concentration	Total Population Screened ^a	Number of Convertants ^b		Convertants Per 10 ⁵ Survivors	
					Ade ⁺	Try ⁺	Ade ⁺	Try ⁺
TC	Li	FDA 71-36	H	2.24 (84)	110	14	49.11	6.25
		FDA 71-36	L	3.62 (136)	129	19	35.64	5.25
	Lu	FDA 71-36	H	3.75 (141)	106	10	28.27 (c)	2.67
		FDA 71-36	L	3.28 (123)	126	16	38.41	4.88
	T	FDA 71-36	H	3.35 (126)	95	13	28.36	3.88
		FDA 71-36	L	3.01 (113)	84	2	27.91 (c)	0.66

NOTE: TC = test compound
 H = high dose
 L = low dose
 Li = liver
 Lu = lung
 T = testes
 a = number x 10⁵
 b = number at 10⁻¹ dilution
 (c) = contamination present
 () = percent survival

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BIONETICS

ACTIVATION SUSPENSION TESTS
WITH SACCHAROMYCES INDICATOR STRAIN D4:
POSITIVE AND SOLVENT CONTROL RESULTS

SPECIES: MONKEY

DATE: 9-20-74

Strain D4

Test	Organ	Compound	Concentration	Total Population Screened ^a	Number of Convertants ^b		Convertants Per 10 ⁵ Survivors	
					Ade ⁺	Try ⁺	Ade ⁺	Try ⁺
PC	Li	DMNA	150 μ moles/ml	5.67	73	68	12.87	11.99
	Lu	DMNA	150 μ moles/ml	5.46	25	21	4.58	3.85
	T	DMNA	150 μ moles/ml	4.52	16	21(c)	3.54	4.65
SC	-	DMNA	150 μ moles/ml	5.45	25	21	4.59	3.85
	-	SALINE	-	5.46	9	15(c)	1.65	2.75

NOTE: PC = positive control
 SC = solvent and chemical controls
 DMNA = dimethylnitrosamine
 Li = liver
 Lu = lung
 T = testes

a = number x 10⁵
 b = number at 10⁻¹ dilution
 (c) = contamination present

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BIONETICS

Litton

ACTIVATION SUSPENSION TESTS
WITH SACCHAROMYCES INDICATOR STRAIN D4

SPECIES: MONKEY

DATE: 9-20-74

Strain D4

Test	Organ	Compound	Concentration	Total Population Screened ^a	Number of Conyertants ^b		Conyertants Per 10 ⁵ Survivors	
					Ade ⁺	Try ⁺	Ade ⁺	Try ⁺
TC	Li	FDA 71-36	H	5.97 (109)	15	16	2.51	2.68
		FDA 71-36	L	5.82 (106)	27	11	4.64	1.89
	Lu	FDA 71-36	H	7.17 (131)	26	14	3.63	1.95
		FDA 71-36	L	5.08 (93)	28(c)	12	5.51	2.36
T		FDA 71-36	H	6.01 (110)	29	16	4.83	2.66
		FDA 71-36	L	5.43 (99)	19	0	3.50	-

NOTE:

TC = test compound
H = high dose
L = low dose
Li = liver
Lu = lung
T = testes
a = number x 10⁵
b = number at 10⁻¹ dilution
(c) = contamination present
() = percent survival

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BIONETICS

XII. SUMMARY OF RESULTS AND INTERPRETATION

Compound FDA 71-36, calcium propionate, was evaluated for possible mutagenic or recombinogenic activity in Salmonella and Saccharomyces. The results of this evaluation are as follows:

A. Salmonella Test Results

1. Plate tests

None of the plate tests indicated mutagenic activity against the three tester strains at a dose of 0.5% added to the surface of a lawn of bacteria or spread with the lawn of bacteria plus organ homogenates for activation. No zone of toxicity was observed in these assays.

2. Non-activation suspension tests

The results of these tests were negative. The high dose data from TA-1538 was obtained from a separate test.

3. Activation suspension tests

All tests were negative with mouse, rat and monkey organ homogenates.

B. Saccharomyces Test Results

1. Non-activation suspension tests

The results of these tests were negative. The frequencies of ade⁺ convertants were about 10-fold higher than the try⁺ frequencies. This relationship is often found and indicates that the adenine heteroalleles are somewhat less stable than the tryptophan alleles. The two frequencies can be made more similar with stringent selection procedures but we have found that the values obtained in this study are sufficient to detect induced changes.

2. Activation suspension tests

The results obtained using rat organ homogenates showed slightly elevated conversion frequencies at the high dose using liver activation preparations. These increases were not considered significant. All other data from the activation tests were clearly negative.

C. Additional Comments

It can be seen that the spontaneous reversion frequencies for the indicator strains are quite variable. This was expected and has been reported in the literature for both the Salmonella and yeast strains. Although cultures with quite low spontaneous frequencies can be selected, we feel that ranges of $1-5 \times 10^{-8}$ for TA-1535 and $5-10 \times 10^{-8}$ for TA-1537 and TA-1538 are the mode.



The survival ranges, although not recorded, are also quite variable ranging from over 100% to less than 50% of the control values. It is our feeling that survival values are dependent on many factors and not only the toxicity of the test compound as determined in the initial survival curves.

Submitted by:

David Brusick, Ph.D.

David Brusick, Ph.D.
Director, Department of Genetics



BIONETICS

APPENDIX

SUMMARY OF TESTS EVALUATING DMSO FOR GENETIC
ACTIVITY IN SALMONELLA AND SACCHAROMYCES



BIONETICS

COMPOUND DIMETHYSULFOXIDE

A. Suspension Tests

Test	<u>Activation</u>		<u>Salmonella Reversion Frequencies (x 10⁻⁸)</u>		<u>Saccharomyces D4 Conversion Frequencies (x 10⁻⁵)</u>	
	Species ^a	Organ ^b	TA-1535	TA-1538	Ade ⁺	Try ⁺
<u>Non-activation</u>						
Control (-C)	-	-	0.74	0.88	32.51	4.34
High Dose ^c	-	-	1.91	1.05	28.32	2.95
Low Dose ^d	-	-	0.53	1.37	40.73	0.49
<u>Activation</u>						
Control (+C)	-	-	1.80	0.36	38.27	2.47
Control (-C)	-	-	1.43	1.04	37.12	2.64
High Dose ^c	M	Li	0.34	1.07	47.77	2.75
	M	Lu	0.59	0.58	36.29	1.39
	M	T	0.62	0.30	34.35	1.94
Low Dose ^d	M	Li	-	0.87	34.02	1.18
	M	Lu	0.43	3.14	42.30	1.40
	M	T	0.11	0.39	45.95	2.32

Note: (-C) = solvent control and (+C) = test chemical control without homogenate

a M = mouse
Mo = monkey
R = rat

b Li = liver
Lu = lung
T = testes

c Bacteria = 3%
Yeast = 5%

d Bacteria = 1.5%
Yeast = 2.5%

COMPOUND DIMETHYSULFOXIDE

B. Plate Tests

Test	<u>Activation</u>		<u>Salmonella Responses</u>		
	Species ^a	Organ ^b	TA-1535	TA-1537	TA-1538
<u>Non-activation</u>					
Control (-C)	-	-	-	-	-
Test compound (3%)	-	-	-	-	-
<u>Activation</u>					
Control (+C)	-	-	-	-	-
Control (-C)	-	-	-	-	-
Test compound (3%)	M	Li	-	-	-
	M	Lu	-	-	-
	M	T	-	-	-
	R	Li	-	-	-
	R	Lu	-	-	-
	R	T	-	-	-
	Mo	Li	-	-	-
	Mo	Lu	-	-	-
	Mo	T	-	-	-

Note: (-C) = solvent control and (+C) = chemical control without homogenate

a M = mouse
Mo = monkey
R = rat

b Li = liver
Lu = lung
T = testes